General information	
PPP-number	TKI-AF-16012
Title	*Nutrition to improve quality of life of IBS patients*
Theme	Nutrition & Health
Implementing institute	Wageningen Food & Biobased Research
Project leader research (name + e-mail address)	Coen Govers – coen.govers@wur.nl Nicole de Wit – nicole.dewit@wur.nl
Coordinator (on behalf of private partners)	Saskia van Hemert – Winclove Probiotics
Project-website address	https://www.wur.nl/nl/project/Voeding-die-bijdraagt-aan-een-betere-kwaliteit-van-leven-van-mensen-met-een-prikkelbare-darm.htm; https://www.ibsqutrition.nl/nl/ibs.htm
Start date	01-04-2017
Final date	31-10-2021

Approval by the coordinator of the consortium The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report.	
Assessment of the report by the coordinator on behalf of the consortium:	X **Approved** ☐ Not approved
Additional comments concerning the annual report:	All deviations of the original plan are well discussed and all partners can give input on the plans.

Summary of the project	
Problem definition	Irritable Bowel Syndrome (IBS) is a disease that affects a large number of people. It is estimated that 10-20% of the total world population have mild or severe IBS symptoms reducing their quality of life. To date, no adequate treatment is available. This is partially due to the heterogeneity of the patients and the complicated pathology in which not all mechanisms are understood. IBS is a multifactorial disease in which the intestinal cell wall, immune system, enteroendocrine cells and the microbiota all have an important role. According to gastroenterologists, patients themselves often report that the changes in diet have the most pronounced beneficial effects on their IBS symptoms but it is not known for what subgroup of patients this is true, what the mechanism behind it is and what kind of nutrition has the biggest impact.
Project goals	The 3 main objectives of this research are: 1. Increase insights into the mechanisms behind the IBS pathology and how food can have an influence on this. 2. Identify (new) links between nutritional compounds and/or food patterns and relief of IBS symptoms for certain subgroups taken the heterogeneity of the patients into account. 3. Develop and optimize *in vitro* models to serve as a screening tool for future studies towards IBS relieving nutrition. The research is divided into 3 work packages: Work package (WP) 1 focuses on the development of the *in vitro* models that mimic IBS-related symptoms with individual and combined cell cultures. These *in vitro* models are employed to screen food components from industrial partners to identify those that might relief IBS symptoms. In WP 2 an

	animal study and human trial will be performed to validate the *in vitro* tool box for the identification of food components to treat IBS symptoms and validate specific compounds for their effect on these symptoms. In WP 3 the nutritional habits of IBS patients and the potential dietary changes that they already have made to deal with their symptoms are investigated and this is linked to improved quality of life and microbiota changes. All work packages contribute to gaining more insight into the mechanisms behind the IBS pathology.

Results	
Planned results 2019	**WP1: this work package comprises the setup of various cell models that relate to IBS symptoms and provide relevant read-outs towards the impact of food compounds** Immune activation: After testing many different stimuli, incubation periods and read-outs we unfortunately had to conclude that the **LUVA mast cell** line was not reliable in its responses to IBS-relevant stressors such as CRH and inflammatory cocktails and therefore unfit as in vitro model for this project. As we at the very start of the project already compared different mast cell lines and concluded this LUVA cell line was the only feasible cell line to test, we were forced to abandon this line of research. The second immune activation goal was to induce **activated PBMC** responses that could be linked to and impaired barrier of Caco-2 and identify modification of responses and effects on Caco-2 barrier by test compounds. We analysed the medium of PBMCs after stimulation with Con-A for immune components, and did so using 16 different donors for the PBMCs. The medium from PBMCs after Con-A stimulation was also used to damage the barrier of Caco-2. Both read-outs (barrier and immune components) were correlated, which revealed that granzyme B, Perforin and sFas/sFasL were correlated to barrier integrity, not IFNg. A mixture of medium from ConA-stimulated PBMCs from different donors was and will now be used to challenge Caco-2 and identify if nutritional compounds have an protective effect on barrier function. Intestinal barrier (Caco-2): Previously we have set up a model to measure fat-induced **LPS translocation** in Caco-2. LPS translocation could instigate low grade inflammation. We finished analysis whether the nutritional compounds were capable of reducing LPS translocation in Caco-2. We also started analysing the expression pattern of a selected subset of **genes in Caco-2** exposed to nutritional compounds. We analyzed IL-8, relating to immune signaling, and PRSS3 and SERT, relating to visceral hypersensitivity, in biological duplicate experiments and are currently conducting n=3. Serotonin production (BON-1): We optimized the BON-1 model to study CRH-induced serotonin production, linked to stress-induced hypersensitivity in IBS patients. Upon developing this model during the last period we performed and finished experiments to identify if nutritional compounds can modify the CRH-induced serotonin production by BON-1 cells. Microbial metabolites from small batch fermentation: Batch fermentation experiments were performed to study the effect of nutritional compounds on microbiota activity and microbial metabolites. The measurements of pressure, pH and SCFA analysis were complemented with tryptophan and ammonia quantification in supernatant of the fermentations. Both are considered as metabolites that might aggravate IBS symptoms. As additional output from those measurements we also gained data on all free amino acids.

WP2: this work package consists of in vivo studies to further test and validate the food compounds for their effects on IBS symptoms and QOL.

Animal study:
Last year we spend a considerable amount of time on drafting, revising and amending the application for approval of the animal study by the ethical committee (CCD). Unfortunately, the CCD (ethical committee) did not see the urgency and value of this new food-related mouse model for IBS. They especially questioned the ability to translate the results of this mouse model to human relevance. Within the time frame of our project we do not have the capacity to accurately validate this mouse model for human relevance, and so we had to decide to withdraw the animal study from our project. This disappointing process already has led to a considerable delay in the project (see mutation form submitted in 2019) and now also results in a significant change in the project plan.
We are currently in the process of designing alternative studies that compensate for or are acceptable alternatives to the read-outs of the animal trial.

Human trial:
Due to a delay in the process to obtain approval for the animal trial and the unforeseen necessity now to find an alternative for the animal study, the human trial is now scheduled for spring of 2021. However, we have already initiated discussion with the project partners on wishes, necessities and feasibility of the trial.

WP3: This work package covers the investigations towards the nutritional habits of IBS patients linked to their quality of life and microbiota changes.

An online survey was performed, which assessed of 1601 IBS patients self-reported complaints on 44 pre-selected dietary triggers after eating. From this data, we did not find a difference in self-reported complaints between the IBS subtypes (constipation, diarrhoea, alternating or unspecified) or severity classes of IBS (mild, moderate or severe). Response to greasy foods, onions, cabbage, spicy and fried foods are mentioned most often as causing complaints. Patients with more severe IBS, and who experience anxious or depressive symptoms tend to respond severe to more dietary triggers. A manuscript is now submitted for publication.

For the microbiota-IBS severity study, 100 IBS patients and 30 healthy controls were recruited. After the first data collection point (T1), the 30 most mild and 30 most severe IBS patients were included for data collection at T2 (after one month). All subjects are aged between 18 and 65 years. Faeces was collected for microbiota composition and metabolite profiling. In addition, dietary intake, Quality of Life, depression and anxiety scores, as well as stool consistency and frequency, were assessed by validated questionnaires. First analyses indicate that some microbial taxa, among others bifidobacteria differ between healthy controls and IBS patients. Moreover, metabolite profiles as well as severity scores vary between subjects as well as within the same subject over time. A manuscript will be prepared.

Achieved results 2019	**Finished in 2019**: Deliverable 1 - Analyse compounds for their effects on the Caco-2 barrier integrity analysed impact of compounds on healthy and challenged intestinal barrier measuring TEER and gene expression. Deliverable 2 – analyse compounds for their (indirect) effects on CRH-induced tryptase and histamine release by mast cells Demonstrated unfeasibility of use of mast cells Deliverable 3 – Questionnaire within IBS patient population Finished previous year. Finalized microbiota and SCFA profilling in microbiota study with analysis ongoing Deliverable 4 – analyse compounds for their effects on serotonin production and secretion by enterochromaffin cells test enterochromaffin (BON-1) cells for CRH-induced serotonin production and how compounds might modify this response Deliverable 5 – analyse compounds for their (indirect) effects on IFNg-release by PBMCs Demonstrated that proposed model of CRH-induced IFNg release and IFNg-mediated barrier disruption were not feasible. However, efforts on alternative means to include immune read-outs in the in vitro platform are in progress Deliverable 6 – analyse effects of compounds on microbiota composition and SCFA production analysed tryptophan and ammonia (and free AA) to complete small batch fermentation experiments and analysis Deliverable 7 – Perform Mice experiment to further screen compounds for use in human study Cancelled; see below
Planned results 2020	Deliverable 7: the animal study will be cancelled but we are deliberating what study should replace this. This study should be performed in 2020 and be budget-neutral. Deliverable 8 – perform human study to validate in vitro and mice trial data as pre-screenings tool the METC application should be (almost) finished and patients recruitment should be initiated for the human study in 2020 to be able to perform the study in Q1/2 of 2021. Publications on in vitro studies, Questionnaire study, Microbiota study

Deliverables/products in 2019 (provide the titles and /or a brief description of the products/deliverables or a link to a website.
Scientific articles:
External reports: Title: Dietary triggers in Irritable Bowel Syndrome are not related to subtypes or severity Authors: Iris Rijnaarts, Nicole de Wit, Erwin Zoetendal, Coen Govers, Ben Witteman, Nicole de Roos Orally presented at - Donderdag 24 oktober bij McGill University, Sainte-Anne-De-Bellevue, Canada - Woensdag 30 oktober bij Waterloo University, Waterloo, Canada

- Dinsdag 10 december bij Food For Thought, Alliantie Gelderse Vallei, Ede
Articles in professional journals/magazines:
(Poster) presentations at workshops, seminars, or symposia.
TV/ radio / social media / newspaper:
Remaining deliverables (techniques, devices, methods, etc.): - Free amino acid measurement in fermentation supernatant - BON-1

https://topsectoragrifood.nl/project/af-16012-nutrition-to-improve-quality-of-life-of-ibs-patients/

https://www.wur.nl/en/project/Food-that-contributes-to-a-better-quality-of-life-for-people-with-an-irritable-bowel.htm